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Cadmium, Copper, Nickel, and Zinc Availability in a Biosolids-Amended Piedmont Soil Years after Application

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ABSTRACT

Concerns over the possible increase in phytoavailability of biosolids-applied trace metals to plants have been raised based on the assumption that decomposition of applied organic matter would increase phytoavailability. The objectives of this study were to assess the effect of time on chemical extractability and concentration of Cd, Cu, Ni, and Zn in plants on plots established by a single application of biosolids with high trace metals content in 1984. Biosolids were applied to 1.5 by 2.3 m confined plots of a Davidson clay loam (clayey, kaolinitic, thermic Rhodic Kandiudults) at 0, 42, 84, 126, 168, and 210 Mg ha^{-1}. The highest biosolids application supplied 4.5, 760, 43, and 620 kg ha^{-1} of Cd, Cu, Ni, and Zn, respectively. Radish (*Raphanus sativus* L.), romaine lettuce (*Lactuca sativa* L. var. *longifolia*), and barley (*Hordeum vulgare* L.) were planted at the site for 3 consecutive years, 17 to 19 yr after biosolids application. Extractable Cd, Cu, Ni, and Zn (as measured by DTPA, $CaCl_2$, and Mehlich-1) were determined on 15-cm depth samples from each plot. The DTPA-extractable Cu and Zn decreased by 58 and 42%, respectively, 17 yr after application despite a significant reduction in organic matter content. Biosolids treatments had no significant effect on crop yield. Plant tissue metal concentrations increased with biosolids rate but were within the normal range of these crops. Trace metal concentrations in plants generally correlated well with the concentrations extracted from soil with DTPA, $CaCl_2$, and Mehlich-1. Metal concentrations in plant tissue exhibited a plateau response in most cases. The uptake coefficient values generated for the different crops were in agreement with the values set by the Part 503 Rule.

Repeated land application of biosolids increases heavy metal concentration of soils (Sloan et al., 1998), which can result in an increase in trace metal uptake into crop tissue (Corey et al., 1987; Berti and Jacobs, 1996). Plant uptake is one of the major pathways by which biosolids-borne potentially toxic trace metals enter the food chain (Chaney, 1990).

The USEPA 40 CFR Part 503 "Standards for the Use and Disposal of Sewage Sludge" (USEPA, 1993) employed a risk assessment methodology (USEPA, 1992) to establish trace elements standards for land-applied biosolids. This rule considers the exposure of humans, animals, and plants to biosolids trace metals through 14 possible pathways. Each element has a reference pollutant load calculated for each pathway to avoid detrimental effects to highly exposed individuals (USEPA, 1995). The limiting value for a particular metal is its smallest reference pollutant load. This, in turn, permitted the calculation of allowable pollutant concentration in biosolids for each trace element (USEPA, 1993). The Part 503 rule permits application of biosolids' trace elements to agricultural land until the Cumulative Pollutant Loading Rate for the most restrictive trace element is reached.

The protectiveness of the Part 503 rule has been questioned over some assumptions made in the underlying risk assessment (McBride, 1995; Harrison et al., 1997). Change in pollutant bioavailability after termination of biosolids application and the relationship between plant metal uptake and time after application and metal loading are still contentious. Harrison et al. (1997) argued that the USEPA analysis did not adequately consider the variability in plant accumulation and soil sorption capacity of trace metals. The uptake coefficient (UC)—the amount of a metal assimilated by a plant relative to the amount applied to the soil—is critical to a number of the pathways in the risk assessment. The use of geometric means to generate the UC for different crops has been criticized as oversimplified (Harrison et al., 1997). This has led some researchers to voice concerns that the geometric mean UC values used in the risk assessment are low, especially for acid soils which increase uptake of trace element cations (Harrison et al., 1997; Chaney and Ryan, 1994).

Long-term availability of biosolids-applied trace metals to soils has been another issue of concern. Most of the data used to develop the Part 503 rule were from studies in which trace metal uptake by plants was measured immediately following or within a few years after biosolids application. Some researchers contend that the organic matter component of biosolids is the primary factor controlling availability. Concerns have been raised that the availability of added trace metals may increase after termination of biosolids applications due to the decomposition of organic matter (Beckett et al., 1979; McBride, 1995). The risk assessment conservatively assumes that the relationship between metal added in biosolids and plant uptake will be linear despite data that suggest that uptake will attain a plateau response. The observed uptake patterns may be a result of the adsorptive inorganic components (Fe, Mn, and Al oxyhydroxide minerals) added to soils with the biosolids (Corey at el., 1987; Hettiarachchi et al., 2003). According to the "plateau effect," the rate of trace metals uptake decreases as the biosolids-applied trace metal

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Abbreviations: ADSS, aerobically digested sewage sludge; ANOVA, analysis of variance; CEC, cation exchange capacity; DTPA, diethylenetriaminepentaacetic acid; EQ, exceptional quality; ICP-AES, inductively coupled plasma–atomic emission spectrometer; LSD, least significant difference; NPAREC, Northern Piedmont Agricultural Research and Extension Center; OM, organic matter; UC, uptake coefficients; USEPA, United States Environmental Protection Agency; VCE, Virginia Cooperative Extension.

concentrations in soils increase (USEPA, 1993). It is crucial to understand the long-term effects that application of biosolids has on metal availability.

Various chemical reagents have been used to estimate the fraction of trace metals that are potentially available to plants. The most frequently employed reagents are chelating agents such as diethylenetriaminepentaacetic acid (DTPA) (Lindsay and Norvell, 1978). Others utilized nonbuffered salt solutions such as $CaCl_2$, $Ca(NO_3)_2$, and NH_4NO_3, and Mehlich-1 to estimate metal availability. Diethylenetriaminepentaacetic acid was developed to detect potential deficiencies, whereas the salt extracts were developed to measure potentially excessive bioavailable trace metals. Diethylenetriaminepentaacetic acid has often proven effective for assessing metal availability to plants. Trace metals extracted by DTPA are shown to correlate with plant metal uptake if soil pH variation of the treated soils is small (Bidwell and Dowdy, 1987; Sommers et al., 1991). Nonbuffered salt solutions, such as $CaCl_2$, are now being widely used (Eriksson, 1990; Novozamsky et al., 1993) and have been found to correlate closely with plant uptake (Hooda et al., 1997; Sanders and Adams, 1987). Mehlich-1 and complexing extractants like DTPA tend to extract more metals from soil than neutral salts solution and, thus, overestimate the amounts of metals immediately available to plants.

The objectives of our research were (i) to evaluate the long-term availability of biosolids-applied trace metals in a weathered piedmont soil; (ii) to calculate and evaluate the UC values of Cd, Ni, Cu, and Zn for radish, romaine lettuce, and barley; and (iii) to determine the plant tissue metal uptake response of biosolids derived trace metals.

MATERIALS AND METHODS

Field Plots and Treatments

A field test was established in the spring of 1984 to evaluate the use of aerobically digested sewage sludge (ADSS) from a wastewater treatment plant with a major industrial input on cropland (Rappaport et al., 1988). The experiment was conducted at the current site of the Northern Piedmont Agricultural Research and Educational Center (NPAREC) in Orange, VA, on a Davidson clay loam. Soil chemical properties of relevance before biosolids application included a cation exchange capacity of 12.5 $cmol_c\ kg^{-1}$, organic matter content of 18 $g\ kg^{-1}$, and a pH of 5.7.

Field experimental plots were constructed to prevent the lateral movement of biosolids constituents. The plots consisted of an isolated volume of soil, 2.3 by 1.5 m and 0.9 m high. Isolation of this soil volume was accomplished by excavating a trench 20 cm wide and 0.9 m deep and wrapping these soil blocks with 254-micrometer polyethylene film. Connection of the plastic wraps to wooden (5 cm wide by 20 cm deep) boards around the plots above the ground ensured total lateral isolation (i.e., prevented lateral movement and runoff). An aerobically digested biosolids was applied at rates of 0, 42, 84, 126, 168, and 210 dry $Mg\ ha^{-1}$ in spring 1984. The treatments were arranged in a randomized complete block design with four replicates.

The biosolids contained considerably higher concentrations of trace metals than are currently found in typical land-applied

Table 1. Quantity of biosolids, trace metals, and total N and P applied at the NPAREC study site (Rappaport et al., 1988).

Biosolids rates	N	P	Cd	Cu	Ni	Zn
$Mg\ ha^{-1}$	$kg\ ha^{-1}$					
42	670	1380	0.9	152	8.6	124
84	1340	2760	1.8	304	17.2	248
126	2010	4140	2.7	456	25.8	372
168	2680	5520	3.6	608	34.4	496
210	3350	6900	4.5	760	43.0	620
CPLR†			39	1500	420	2800

† CPLR, USEPA Part 503 Cumulative Pollutants Loading Rate.

biosolids. The applied ADSS has 21.5 $mg\ kg^{-1}$ Cd, 3650 $mg\ kg^{-1}$ Cu, 210 $mg\ kg^{-1}$ Ni, and 2980 $mg\ kg^{-1}$ Zn. By comparison, trace metals levels in biosolids from the same treatment plants averaged 0.4 $mg\ kg^{-1}$ Cd, 138 $mg\ kg^{-1}$ Cu, 7.6 $mg\ kg^{-1}$ Ni, and 299 $mg\ kg^{-1}$ Zn in 2004. Copper and Zn concentrations were above the exceptional quality (EQ) limits for pollutant concentration biosolids (Table 3 of Section 503.13, USEPA, 1993); thus, Part 503 would require lifetime loading rate of this biosolids to be tracked and limited. The 210 $Mg\ ha^{-1}$ biosolids rate supplied 4.5 $kg\ ha^{-1}$ Cd, 760 $kg\ ha^{-1}$ Cu, 43 $kg\ ha^{-1}$ Ni, and 620 $kg\ ha^{-1}$ Zn (Table 1). This rate provided 11.5% of the Cd limit, 50.7% of the Cu limit, 10.2% of the Ni limit, and 22.1% of the Zn limit.

Plot Management

The experimental plots had been planted to Pioneer 3193 field corn annually for the period from 1984 to 2000. Plots were roto-tilled every spring in preparation for planting. All plots received the same rates of N fertilizer according to Virginia Cooperative Extension (VCE) guidelines (Donohue and Heckendorn, 1994). Phosphorus and K fertilizers were applied according to VCE soil testing recommendations. Pest control and weeding were performed according to VCE recommendations (VCE, 1992). The aboveground portion of the crop was totally removed at physiological maturity. Lime applications in 1989 and 1998 were made to adjust the pH to 6.

Cropping System

Three years of cropping was initiated in the spring of 2001 to assess the phytotoxicity of Cu, Ni, and Zn and the phytoaccumulation of Cd, Cu, Ni, and Zn by barley, radish, and romaine lettuce cultivar Paris Island Cos. Barley was planted in late October 2001 only, and the radish and lettuce were planted in early April 2001, 2002, and 2003. The plots had been limed to a pH of approximately 6.0 in May 1998 to minimize acid pH-induced Al, Cu, and Zn phytotoxicity and trace metal uptake. The change in soil pH during the duration of the study is presented in Table 2. Plots were irrigated regularly to avoid moisture stress. Commercial fertilizer N (as ammonium nitrate) at 120 $kg\ N\ ha^{-1}$, P (as triple superphosphate) at 22 $kg\ P\ ha^{-1}$, and K (as muriate of potash) at 83 $kg\ K\ ha^{-1}$ were applied to each plot every spring as required according to the Virginia Cooperative Extension (VCE) soil testing recommendations (Donohue and Heckendorn, 1994).

Plant Analysis

The plants were harvested when physiologically mature. Radish tops were separated from globes, and radish and lettuce samples were washed thoroughly with running tap water and rinsed three times with double-deionized water. The plant tissues were dried in a forced air oven at 70°C for 72 h or until constant mass was achieved. Dry weight was measured by weighing dry subsamples of 6 lettuce and 16 radish plants

Table 2. Soil pH (avg. of four replicates) in the biosolids-amended soil experiment from 2001 to 2003. SD in parentheses.

	pH		
Biosolids rates	2001	2002	2003
Mg ha^{-1}			
0	5.8 (0.22)	5.7 (0.06)	5.4 (0.23)
42	5.9 (0.17)	5.7 (0.13)	5.4 (0.20)
84	6.0 (0.24)	5.6 (0.21)	5.3 (0.27)
126	6.0 (0.24)	5.8 (0.13)	5.3 (0.21)
168	5.9 (0.22)	5.6 (0.12)	5.3 (0.10)
210	6.0 (0.30)	5.6 (0.09)	5.5 (0.11)

randomly chosen. Crops were visually monitored throughout the growing season for any sign of deficiency or toxicity.

Dried samples were ground in a stainless steel Wiley mill to pass a 0.5-mm sieve in preparation for chemical analysis. Ground samples were stored in paper bags and placed in the oven at 65°C to remove any moisture added during grinding and handling of the samples. Plant tissue was digested using a nitric acid microwave method. A 0.5-g aliquot of each ground sample was weighed and placed in a digestion vessel to which 10 mL of trace metal grade HNO_3 acid was added. Vessels were tightly closed and transferred to the Ethos Plus 800 Microwave Labstation (Milestone Microwave Lab Systems, Germany). Digestion followed a three-step program. The first stage program brought the sample to 140°C in 5 min, the second stage permitted a slow rise of temperature to 190°C in 10 min, and the third stage allowed the digest to remain at 190°C for an additional 10 min. Reagent blanks, laboratory standards, and NIST (1573a) standard samples (NIST, 2001) were routinely included in the analysis. Samples were analyzed for Cd, Cu, Ni, and Zn using a Thermo Jarrell Ash (Fitchburg, MA) inductively coupled argon plasma–atomic emission simultaneous spectrometer (ICAP-AES).

Soil Analysis

Soil samples were collected from the top 15 cm of each plot before planting. Samples were air-dried in clean plastic bags and ground with a glass mortar and pestle to pass a 2-mm sieve. Soil pH was determined in 1:1 soil/water suspension after a 1-hr equilibration period. Soil organic matter was determined by the Walkley-Black method. Mehlich-1 (0.05M HCl and 0.0125 H_2SO_4) (Mehlich, 1953), neutral salts (0.01 *M* $CaCl_2$) (Novozamski et al., 1993), and diethylenetriamine pentaacetic acid extracting (0.005 *M* DTPA, 0.1 *M* TEA, and 0.01 *M* $CaCl_2$, adjusted to pH 7.3) (Lindsay and Norvell, 1984) solutions were employed to extract various soil trace metals fractions as potential indicators of plant-available trace metals. The soil extracts were analyzed for Cd, Cu, Ni, and Zn with an inductively coupled plasma–atomic emission spectrophotometer (ICP-AES).

Statistical Analysis

Plant weight and trace metal concentration data were evaluated by analysis of variance (ANOVA) and by the least significant difference (LSD) mean separation procedures at the 0.05 level of significance (Steele and Torrie, 1980). Linearity of the metal uptake curves was tested using NLIN procedure. Extractable soil trace metals data were also evaluated by LSD mean separation procedures at the 0.05 level of significance. The UC (plant uptake slopes) values were calculated with the linear regression statistical method. The slope of the linear regression line of the metal concentration in plant tissue compared with the amount applied to the soil was taken as the UC for those trace metals. Relationships between plant metal concentrations and DTPA-, Mehlich-1–, and $CaCl_2$–extractable trace metals were determined by Pearson correlation coefficients.

Table 3. Long-term effect of biosolids application on the soil organic matter content.

Biosolids rates	1984	1992	1995	2001
Mg ha^{-1}	organic matter, g kg^{-1}			
0	22e†	22d	22d	21d
42	29d	24d	25d	24d
84	36c	28c	29c	28c
126	49b	33b	32bc	31bc
168	50b	37a	36ab	36ab
210	65a	40a	39a	40a

† Values within columns followed by different letters are significant at the 0.05 probability level.

RESULTS AND DISCUSSION

Soil Chemical Analysis

Soil organic matter (SOM) increased from <20 g kg^{-1} in the control to >60 g kg^{-1} at the high biosolids rate in the year 1984 after biosolids application (Table 3). By 1992, the concentrations of SOM had decreased by 5 and 8 g kg^{-1}, respectively, in the 42 and 84 Mg ha^{-1} treatments, had decreased by about 15 g kg^{-1} in the 126 and 168 Mg ha^{-1} treatments, and had decreased by as much as 25 g kg^{-1} in the 210 Mg ha^{-1} treatment. No change in organic matter content was observed since 1992 despite the continual tillage and annual removal of all plant tissues from the plots. Our results were consistent with those reported by McGrath et al. (2000) and Bidwell and Dowdy (1987), who concluded that the greatest rate of biosolids organic matter mineralization occurs soon after application. Soil organic matter decomposition rate decreases with time, and a portion of the biosolids-applied organic matter can remain in soils for decades before SOM returns to its background levels.

Measurements of DTPA-extractable Cu and Zn have been made over time on these plots. For the initial 2 yr following biosolids application, a linear increase of DTPA-extractable Cu and Zn with increasing biosolids application was reported (Rappaport et al., 1988). The levels of extractable Cu and Zn at the site in 1984 reached 129 and 78 mg kg^{-1} respectively, at the highest application rate (Table 4). Eleven years after biosolids application, Anderson (1997) also reported a linear increase in DTPA-extractable Cu (R^2 = 0.992) and Zn (R^2 = 0.992) concentrations with biosolids application at the site; however, the concentrations of both metals

Table 4. Long-term effect of biosolids application on DTPA-extractable Cu and Zn.

	DTPA-extractable Cu			DTPA-extractable Zn		
Biosolids rates	1984	1995	2001	1984	1995	2001
Mg ha^{-1}	mg kg^{-1}					
0	1.4f†	3.7f	3.2f	1.6f	2.8f	2.7f
42	24.9e	23.1e	12.6e	19.2e	17.2e	9.1e
84	53.0d	44.3d	25.4d	38.9d	33.3d	19.8d
126	73.4c	64.8c	33.7c	52.4c	49.6c	27.9c
168	119.9b	78.7b	43.3b	73.2b	59.5b	35.5b
210	129.4a	92.8a	53.6a	78.2a	69.9a	49.7a

† Values within columns followed by different letters are significantly different at the 0.05 probability level.

Table 5. Effect of biosolids application rate on radish and lettuce dry weight. Values represent the average yield over 2001–2003 growing seasons.

	Biosolids application rates, Mg ha^{-1}					
Plant	0	42	84	126	168	210
	wt., kg ha^{-1}					
Lettuce	801a†	896a	852a	985a	951a	967a
Radish globes	665a	719a	682a	732a	769a	657a
Radish tops	630a	756a	653a	728a	759a	721a

† Rows followed by different letters are significantly different at the 0.05 probability level.

were lower than those extracted in 1984 despite the lower soil pH. This trend in declining extractability with time continued in 2001. Our results have shown a linear increase of Cu (R^2 = 0.997) and Zn (R^2 = 0.996) extracted with DTPA with increasing application rate; however, the amounts we extracted were much lower than the concentrations reported in 1984 (Table 4), which indicates a significant decrease of metal availability with time. The decrease in organic matter content of the surface soil was accompanied by a significant decrease in the amount of extractable Cu and Zn (Table 4), which contradicts the hypothesis that organic matter is the primary constituent controlling biosolids' metal availability. Mehlich-1 and $CaCl_2$ extraction tests were not conducted in the first few years of the experiment so there is no basis to compare Mehlich-1 and $CaCl_2$–extractable metals over time.

Seventeen years after application, the levels of DTPA-extractable Cu and Zn decreased by as much as 50 to 64% and 40 to 52%, respectively. Mass balance calculations at this site showed that >95% of the applied metals remained concentrated in the top 20 cm (Sukkariyah et al., 2005). The decrease in extractability could be attributed to metals reverting to a more recalcitrant form in soil, such as occlusion in Fe-oxides or chemisorption to surfaces. Bioavailability of trace metals in biosolids-amended soil typically has not increased years after land application has ceased (Bidwell and Dowdy, 1987; Hyun et al., 1998). Chang et al. (1987) and Sommers et al. (1991) reported that the highest availability of trace metals occurred during the period immediately following biosolids application. In other studies, the availability of trace metals has been reported to decrease with time as organic decomposition rates decreased (Bidwell and Dowdy, 1987; Walter et al., 2002).

Crop Yield

Biosolids application rate did not affect the yield of lettuce, radish globes, and shoots in any year (Table 5). There were no discernable differences in growth or visual signs of toxicity or deficiency due to treatment in any of the crops, which is further evidence that plant growth was not affected by the biosolids treatment.

Soil Test Extractant Correlations

The biosolids-amended surface soils have significantly higher trace metal concentrations than the control. The concentrations of the trace metals extracted with 0.05 M

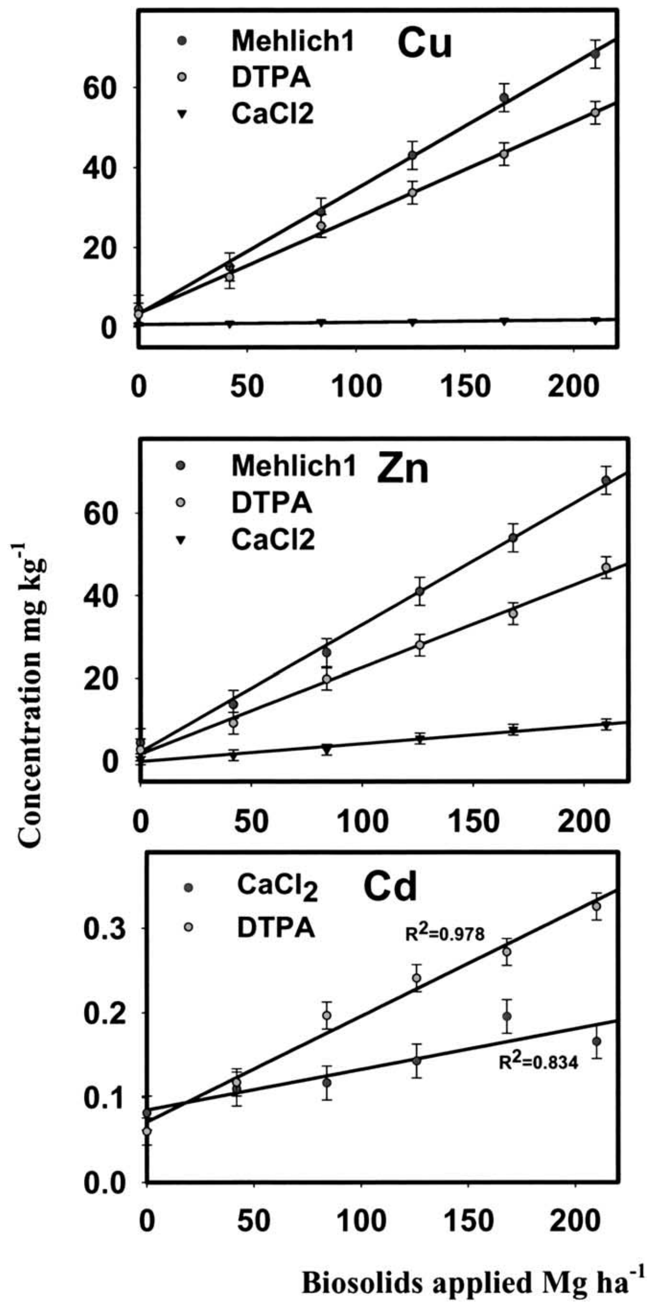

Fig. 1. Effects of biosolids application on the levels of Mehlich-1, DTPA and $CaCl_2$ extractable Zn, Cu, and Cd 17-19 yr after application.

DTPA, 0.01 M $CaCl_2$, and Mehlich-1(0.05 M HCl and 0.0125 M H_2SO_4) increased linearly in response to biosolids additions (Fig. 1). The ability of the extractants to remove metals from the soil decreased in the order: Mehlich-1 > DTPA > > $CaCl_2$ across all rates. Extractable metal concentration was dependent on the total metal content of the soil, which, in addition to pH, are the most important factors in regulating the availability of Cd, Cu, Ni, and Zn to several plant species (Sauerbeck, 1991).

Correlations between the concentrations of Cd, Cu, Ni, and Zn in radish globes and tops, lettuce, and barley and soil metals extracted by 0.05 M DTPA, 0.01 M

Table 6. Pearson correlation coefficients for Cu and Zn uptake by crops and 0.01 M $CaCl_2$, DTPA, and Mehlich-1–extractable soil trace metals.

Extractant	Barley	Radish tops	Radish globes	Lettuce
		Cu Pearson correlation coefficient		
$CaCl_2$	0.510	0.438	0.638	0.361
DTPA	0.660	0.766	0.827	0.008
Mehlich-1†	0.688	0.754	0.874	0.082
		Zn Pearson correlation coefficient		
$CaCl_2$	0.747	0.824	0.848	0.766
DTPA	0.817	0.848	0.881	0.799
Mehlich-1†	0.843	0.854	0.882	0.809

† Mehlich-1 represents averages over 3 yr.

$CaCl_2$, and Mehlich-1 were variable (Table 6). Extractable fractions increased markedly in the biosolids-amended soil as compared with the control. The strength of the correlation between metal concentration in plants and extractable levels in soil was greater for Zn than Cu. Copper and Zn concentrations in plants were better predicted by the Mehlich-1 test, but correlations with $CaCl_2$ and DTPA were also highly significant. There were poor relationships between biosolids treatment and lettuce tissue Cu concentration (Table 6). The DTPA better predicted availability of Cd for lettuce ($R^2 = 0.83$) and Ni for radish globes ($R^2 = 0.77$) than $CaCl_2$. Nickel and Cd concentrations in radish globes and barley were below detection limits. Except for Cu in lettuce, Mehlich-1 consistently correlated with Cu and Zn in each of the crops and, therefore, appears to be a reliable test to assess the metal fraction potentially available to these crops grown at this site. It is important to note that Mehlich-1 is acidic and could dissolve metals from the solid phase that may not be available in the short term.

The concentrations of trace metals extracted with $CaCl_2$ and DTPA were much lower than Mehlich-1–extractable metals. The concentration of the extractant and soil/solution ratio we used may have been low for metal-enriched soils. Norvell (1984) suggested that no more than half the complexing capacity should be used when extracting trace metals with a complexant; otherwise, the concentration will be influenced by the concentration of the other trace metals (Esnaola et al., 2000; Li et al., 2000). Metal exchange with Ca and metal dissolution might have been incomplete due to the low (0.01M) concentration used (Esnaola et al., 2000).

Plant Tissue Uptake Response

The responses of plant tissue trace metal concentrations to biosolids application rate are shown in Fig. 2. Concentrations of the trace metals were plant species-specific. The metal concentrations in all crops were higher in the biosolids treatments than in the control but remained well within the values observed for uncontaminated soils (Kabata-Pendias and Pendias, 1991). None of the trace metals attained toxic concentrations.

The relationships between tissue concentrations and the concentration of metals in soil are shown in Fig. 2 for selected plants and metals (representing the different uptake patterns) and Table 7. Metal concentration in plants displayed a nonlinear plateau response in most cases. The negative coefficients of nonlinearity indicated that an incremental decrease in the uptake rate occurred with increasing biosolids application rate. The response is considered to be linear whenever the coefficient of nonlinearity is nonsignificant. Most of the linear responses were observed for the 2003 growing season, when soil pH was < 5.5.

The increase in Cd and Zn concentrations, with the exception of radish tops in 2001 and 2003, peaked at the high level of biosolids application. Zinc concentration in lettuce increased with biosolids rate and plateaued between 372 and 496 kg Zn applied ha^{-1} (126–168 Mg ha^{-1} biosolids) (Fig. 2a). Lettuce Cd concentration followed a similar pattern (Fig. 2b). Radish globe Zn exhibited a plateau-type response (negative coefficient of nonlinearity) (Table 7), but Zn uptake by radish tops increased linearly in the 2001 and 2003 seasons and attained a plateau in 2002 (Fig. 2c). Barley also showed a plateau-type response (Table 7).

Copper concentration in radish tops (Table 7), barley (Table 7), and radish globes (Fig. 2d) increased with biosolids rate. Lettuce Cu concentration was not increased by biosolids rate. The concentration of Cu in radish globes (Fig. 2d) increased linearly in 2003 but showed a plateau-type response in the other growing seasons. Plateau-type response in the radish tops (Table 7) was observed in 2002 only.

The response to increasing Ni concentrations in soils varied among crops and growing seasons for the same crop (Table 7). Both linear and plateau-type response curves were observed (Fig. 2e). The increase in Ni concentration was linear for the 2003 growing season.

Chaney and Ryan (1992) demonstrated that plant uptake would be lower than predicted by the linear regression model used to formulate the Part 503 rule where plant tissue concentration attains a maximum with increasing biosolids rate (plateau theory). Several studies (Chang et al., 1987; Logan et al., 1997; Brown et al., 1998) have provided evidence that biosolids trace metals uptake by wheat (*Triticum aestivum* L.), corn, and several vegetables reaches a maximum with biosolids application rate. Linear uptake curves were also reported in several studies. For example, Logan et al. (1997) demonstrated that lettuce grown on a silt loam soil that received a one-time application of sewage sludge at rates up to 300 Mg ha^{-1} exhibited linear (decreasing with time) uptake curves.

The reasons for plateau responses have been speculated by many researchers. Corey et al. (1987) suggested that metal uptake would approach a maximum (plateau) at high biosolids metal loadings because the amended soil had higher metal adsorption capacity than the unamended soil. Chaney and Ryan (1993) proposed that increasing biosolids application increases the metal adsorption capacity of soil in addition to soil metal concentration; thus, metal availability at high biosolids application declines as the specific metal adsorption capacity of the amended soil increases (Hettiarachchi et al., 2003).

Plant mechanisms such as exclusion of trace metals, limited transport from root to shoot, and saturation of the carrier system might also contribute to the observed decrease in uptake slope at high metal loadings (Hamon

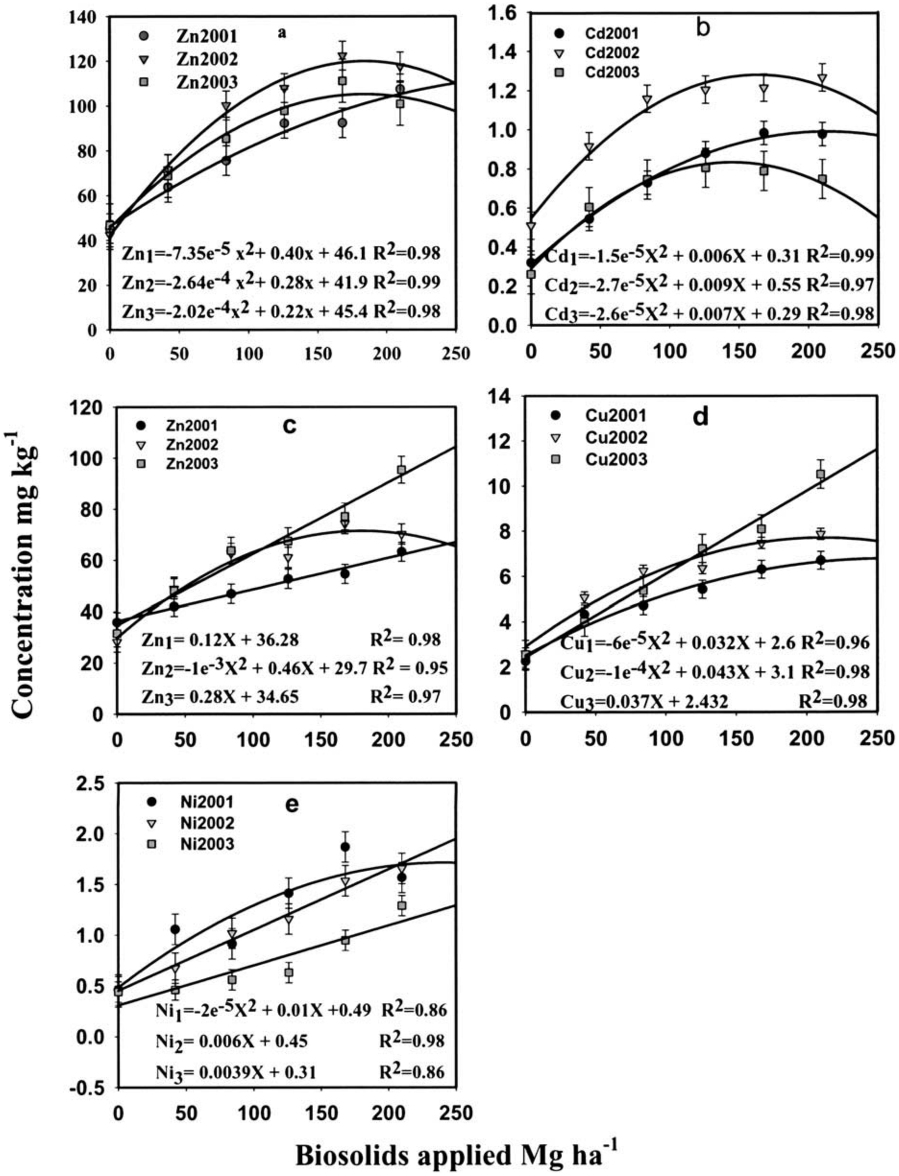

Fig. 2. Trace metals accumulation in plants as a function of biosolids application: (a) Zinc in lettuce; (b) Cd in lettuce; (c) Zn in radish tops; (d) Cu in radish globes; (e) Ni in radish globes.

et al., 1999; McBride, 1995). Hamon et al. (1999) proposed that bioavailability will plateau at some biosolids loading rate and metal uptake by plants will follow the same trend if attenuation of metal concentration is due to biosolids chemistry. Metal concentrations in plants grown at this site displayed a plateau response to trace metals from biosolids applied in most cases. Response was plant species-dependent, metal dependent, and varied among growing seasons. These observations show that in addition to soil reactions, plant uptake control mechanisms play an important role in regulating metal uptake.

Plant Uptake Coefficients

A comparison of the range of UC values calculated for the trace elements in our study and the UC values employed in the USEPA risk assessment is presented in Table 8. The slope of the linear regression curve represents the UC or the efficiency of metal transfer from soil to plants. The UC values estimate the relationship between metal uptake by crops and the amount added to soil. Plants that assimilate greater amounts of metals have higher uptake coefficients.

The UC values of trace metals in our study varied widely among crops. Lettuce had a higher assimilative capacity for uptake of Zn and Cd than other garden crops. The UC for Cd by lettuce was 10 times those for radish globes and tops. Leafy plants like lettuce demonstrate high potential for Cd uptake and transport to edible crop tissues and are the most responsive of the garden vegetables to changes in soil Cd concentration (Brown et al., 1998). The average Cd UC for the crops was in the order: lettuce ≫ radish globes > radish tops. The Zn UC was 1.1 to 2.3 times higher in lettuce than

Table 7. Trace metals concentrations in the dry matters of lettuce, radish globes, and radish tops.

Trace metals	Biosolids application rates, Mg ha^{-1}						
	0	42	84	126	168	210	*a*†
	conc., mg kg^{-1}						
	Lettuce						
Ni							
2002	1.05b‡	1.54b	2.11a	2.24a	2.46a	2.22a	-5.0×10^{-5}
2003	0.34c	0.47bc	0.64b	0.62b	1.16a	1.13a	1.0×10^{-5}NS
	Radish globes						
Zn							
2001	37.8c	50.8b	56.5b	68.1a	71.9a	73.3a	-7.0×10^{-4}
2002	31.8c	40.1c	52.1b	53.0b	62.5a	62.8a	-5.0×10^{-4}
2003	28.7e	43.2d	52.6c	66.5b	76.0a	81.6a	-4.0×10^{-4}
	Radish tops						
Cu							
2001	5.4d	7.1c	6.7c	8.3ab	7.7bc	8.8a	-5.0×10^{-5}
2002	6.8c	9.5b	10.3ab	10.6ab	11.6a	11.3ab	-1.0×10^{-4}
2003	4.6c	5.7bc	6.0b	7.6a	6.8b	8.0a	-4.0×10^{-5}NS
Ni							
2002	1.17c	1.70bc	1.94ab	1.95ab	2.40a	2.17ab	-3.2×10^{-3}
2003	0.47c	0.69c	0.68bc	0.77b	1.09a	1.24a	3.9×10^{-6}NS
	Barley						
Cu	11.1d	15.6c	17.1ab	16.6abc	17.6a	16.3bc	-3.1×10^{-4}
Zn	44.7d	78.4c	85.2bc	90.6b	104a	100a	-1.6×10^{-3}

† Coefficient of nonlinearity; NS means coefficient is nonsigficant as determined by the SAS NLIN PROC and linear uptake curve is assumed.
‡ Row means followed by different letters are significantly different at the 0.05 probability level.

radish. The relative assimilative capacity of the crops in our study for Zn followed the same order as for Cd.

The lowest UC value for all three crops was for Cu, whose uptake in plants to excess concentrations is limited by the soil–plant barrier (McBride et al., 2003). The USEPA concluded that Cu does not pose any significant crop food chain risk to humans (Pathway 1 and 2) (USEPA, 1992); therefore, Cu UC values for these pathways were not computed. Our results supported this conclusion, as UC values for Cu were shown to be very low. Radish globes have the highest Cu UC, followed by radish tops and lettuce.

The Ni UC decreased in the following order: radish globes > radish tops and lettuce. The UC values reported in this study are in agreement with those used in the USEPA risk analysis (Table 8).

CONCLUSIONS

No adverse effects on plant growth or excessive amounts of metal uptake were noted 17 to 19 yr after application despite the high application rate of biosolids that contained concentrations of Cu and Zn that exceeded the pollutant concentration limits. Concentrations in all crops grown at even the highest biosolids rate were well within the sufficiency range observed for agronomic crops (Kabata-Pendias and Pendias, 1991). Our results confirmed that accumulation of trace metals differed widely among plant species. Lettuce accumulated the highest concentrations of Cd and Zn, whereas radish shoots showed the highest increase in Cu concentration.

Available metal concentrations were dependent on the total metal content of soil. The biosolids-amended soils still have significantly higher trace metal concentrations than the control; however, the extractability of the metals has steadily declined during the 17 yr following application despite a significant decline in soil organic matter concentration in the biosolids amended plots. We conclude that the mineralization of biosolids-applied organic matter did not increase availability due to loss in metal binding capacity associated with organic matter as some researchers have proposed.

Metal uptake by plants displayed both plateau- and linear-type responses. Most of the linear responses were observed for the 2003 growing season, when soil pH dropped to below 5.5. Extractable soil trace metals increased linearly with biosolids additions; therefore, it is difficult to conclude whether the plateau response was solely the result of soil metal attenuation or a combination of soil and plant physiological factors. The UC values varied widely among crops as well as among years for a given crop. The UC values determined for lettuce increased in the order: Cu < Ni ≪ Zn < Cd; for radish globes and tops they increased in the order: Cu < Cd < Ni < Zn. The UCs observed for Cd and Zn were higher

Table 8. Comparison of uptake coefficients calculated from our data and those employed by the USEPA for the development of the 503 risk assessment for lettuce and radish.

Trace metals	UC	Avg. UC	USEPA UC†
	Lettuce		
Cd	0.1260–0.167	0.151	0.182
Cu	0.0002–0.001	0.0006	0.012
Ni	0.0005–0.025	0.015	0.016
Zn	0.0960–0.129	0.110	0.125
	Radish globes		
Cd	0.007–0.017	0.011	0.032
Cu	0.005–0.010	0.007	0.012
Ni	0.024–0.029	0.025	0.004
Zn	0.058–0.089	0.073	0.022
	Radish tops		
Cd	0.001–0.017	0.009	0.182
Cu	0.004–0.007	0.005	0.012
Ni	0.001–0.021	0.013	0.016
Zn	0.041–0.096	0.068	0.125

† Average UC used in the USEPA risk assessment analysis.

for lettuce than radish, whereas the opposite was true for Cu. The UCs observed for Ni was very similar for both radish and lettuce. Our UC values agreed with those used in the USEPA risk assessment.

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